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DEVELOPMENT OF AN IMMUNOASSAY FOR BACTERIAL ENDOTOXINS

ANNUAL REPORT

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September 12, 1983

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This report describes our progress in establishing and validating an immunoassay for lipopolysaccharides. The report also describes steps taken to begin to characterize the antigenic site of LPS which is involved in the assay.

1. Assay Development.

1.1. The assay utilizes three sequential steps. In step 1 polystyrene tubes are coated with Re595-LPS, washed with bovine serum albumin (BSA) solution, and dried. In step 2 the sample to be assayed is mixed with ^{125}I-labelled immunopurified anti-(Re595-LPS) IgG and allowed to form soluble antigen-antibody complexes. The mixture is transferred to an LPS coated tube, the uncomplexed ^{125}I-IgG binds to the tube, and the soluble LPS-antibody complexes are washed away. Finally in step 3 the LPS coated tubes with ^{125}I-IgG bound to them are counted to determine the ^{125}I content and thus the amount of bound IgG. These data are then used to prepare a standard curve or to calculate the concentration of LPS in a sample. An example of an Re595-LPS standard curve is shown in Figure 1. For assay development and characterization of assay response to LPS, the most useful quantitative result is the "D-50", defined as the dose of LPS or other substance required to inhibit 50 percent of the maximal binding of anti-(Re595-LPS) IgG. The D-50 value is obtained from a computerized, weighted, least squares fit of all the data to a set of equations found empirically to describe the chemistry of the assay. The mathematical analysis was developed by Dr. D. Rodbard, National Institutes of Health. The program was adapted by us for use with our IBM computer.

1.2. Assay Characterization.

Using purified Re595-LPS, the D-50 obtained is typically 5 nanograms. Since the sample size is 50 microliters, a concentration of 100

nanograms/ml or greater of Re595-LPS is readily detected by the assay. In serum samples it is likely that LPS is present as a complex with high density lipoprotein (HDL) as shown by our studies as well as the studies of others. To determine whether the binding of LPS to HDL would alter the assay response, complexes of Re595 LPS and HDL were prepared and assayed. For complexes prepared in normal serum the D-50 was 4.6 ± 1.3 ng Re595-LPS, while with complexes prepared with HDL in acute phase serum, the D-50 was 2.8 ± 1.1 ng. Thus complexation of the LPS with lipoprotein did not alter the assay response. Undiluted serum is mildly inhibitory in the assay, but a one-fifth dilution of serum, normal or acute phase, is not. Other controls, such as increased lipoprotein concentration and increased non-specific IgG concentration have not shown interference with the assay.

The D-50 values for a variety of LPS other than Re595-LPS have also been obtained and are presented in Table I. These data are preliminary in the sense that the effects of varying methods of sample preparation, the effects of HDL complexation, or the effects of other serum constituents have not been investigated with these different LPS sources. From the D-50 values for the heterologous LPS, the assay is less sensitive than for Re595-LPS although they are all detected. However because this assay system does detect a variety of LPS preparations we are encouraged that the strategy we are pursuing is correct.

2. Immunoreactive fragments of Re595-LPS.

One of the goals of the past year has been to characterize the nature of the determinants in LPS which react with the immunopurified anti-(Re595-LPS) antibody. To do this we have utilized a series of chemical modifications of the Re595-LPS which are known to produce specific chemical alterations. These studies have the potential of yielding a non-toxic immunoreactive fragment of Re595-LPS which would be useful as an immunogen for poly- and monoclonal antibody generation, and for anti-LPS vaccine development.

We have subjected Re595-LPS to acid and alkaline hydrolysis and studied the LPS fragments generated for immunoreactivity using the assay previously described. Hydrolysis in alkali had no effect on the D-50 of Re595-LPS. Alkaline hydrolysis is expected to hydrolyze fatty acid esters present in the lipid A region of LPS but to leave the amide linked fatty acids of the lipid A as well a the lipid A-core linkage intact. These results suggest that the antigenic site of Re595-LPS does not include the ester linked fatty acids of lipid A.

Acid hydrolysis of Re595-LPS did decrease but not eliminate its D-50. Acid hydrolysis is expected to cleave lipid A from the core oligosaccharide and perhaps to cleave the core region as well. Lipid A was isolated from the acid hydrolysed Re595-LPS and was found to be unreactive in the assay. The remaining core fragments were approximately 1 percent as reactive as the parent LPS and these immunoreactive fragments were at least partially dialyzable. Notably, the major sugar of the core, 2-keto-3-deoxy-octulosonate (KDO), was totally unreactive in the assay. These results suggest that the antigenic site of Re595-LPS is some fragment of the core oligosaccharide, is not free KDO and that acid

hydrolysis of Re595-LPS may be a useful first step in generating Re595-LPS fragments for further purification and characterization.

3. Publications Arising During Current Contract.

1. Rao-Bette, M., P.S. Tobias, and R.J. Ulevitch. A versatile anti-core immunoassay for lipopolysaccharides. In preparation.

This paper describes the LPS immunoassay and the results obtained with its use.

2. Tobias, P.S., and R.J. Ulevitch. Control of lipopolysaccharide-high density lipoprotein binding by acute phase protein(s). J. Immunol. In press, 1983.

Patients who may benefit from the assay described will probably be in the acute phase with the concomitant changes in plasma protein levels. This paper is the first in a series to describe the interaction of acute phase serum with lipopolysaccharides.

3. Ulevitch, R.J., P.S. Tobias, and J.C. Mathison. Regulation of the host response to bacterial lipopolysaccharides. Fed. Proc., in press, 1983.

4. Ulevitch, R.J., J.C. Mathison, and P.S. Tobias. The role of the macrophage in the host response to bacterial endotoxins. In: Proc. of the First International Symposium on Pathophysiology of Combined Injury and Trauma, in press, 1983.

5. Ulevitch, R.J. Interactions of bacterial lipopolysaccharides with high density lipoproteins. In: The Cellular Biology of Endotoxin. Elsevier Pub. Co., In press, 1983.

TABLE I
D-50 VALUES FOR VARIOUS LPS TYPES

Organism	D-50 (ng)	
Salmonella minnesota Re595	5.9 6.4 2.0	Ave: 4.7 ± 2.4
Escherichia coli 0111:B4	44.8 14.8 23.0	Ave: 27.5 ± 15
Escherichia coli K235	80 28.7 34.5	Ave: 47.7 ± 28
Salmonella minnesota 9700	136 190 100	Ave: 142 ± 45
Klebsiella pneumoniae	239 98 131	Ave: 156 ± 73
Pseudomonas aeruginosa	147 340	Ave: 243 ± 136

FIGURE 1: STANDARD CURVE FOR Re595 LPS.